

AD _____

Award Number: DAMD17-98-1-8295

TITLE: Targeted Therapy of Human Breast Cancer by 2'-5'A-Antisense
Directed Against Telomerase RNA

PRINCIPAL INVESTIGATOR: John K. Cowell, Ph.D.

CONTRACTING ORGANIZATION: The Cleveland Clinic Foundation
Cleveland, Ohio 44195

REPORT DATE: September 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.

REPORT DOCUMENTATION PAGE

*Form Approved
OMB No. 074-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED	
	September 2001	Annual (1 Sep 00 - 31 Aug 01)	
4. TITLE AND SUBTITLE		5. FUNDING NUMBERS	
Targeted Therapy of Human Breast Cancer by 2-5A-Antisense Directed Against Telomerase RNA		DAMD17-98-1-8295	
6. AUTHOR(S) John K. Cowell, Ph.D.			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Cleveland Clinic Foundation Cleveland, Ohio 44195 E-Mail: John.Cowell@RoswellPark.org		8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES		20011130 048	
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE
13. Abstract (Maximum 200 Words) <i>(abstract should contain no proprietary or confidential information)</i> Targeting telomerase RNA (hTR) for degradation by RNaseL in breast cancer cells using antisense molecules linked to 2-5A has demonstrated high levels of cytotoxicity <i>in vivo</i> and <i>in vitro</i> . The antisense molecules used in preliminary studies carried a phosphodiester backbone which can be recognized by endogenous nucleases and so make the half-life of these molecules very short. Having demonstrated <i>in vitro</i> that breast cancer cells undergo apoptosis following this treatment, we now sought to improve the stability of the antisense molecules by modifying the backbone. The phosphodiester linkages were therefore replaced with thioate or 2'-O-methyl linkages in various combinations. Many of these modifications resulted in the loss of ability to induce apoptosis in breast cancer cells. Although some of the modified oligonucleotides showed reasonable degrees of cytotoxicity, none of these were as efficient as the original (H1) phosphodiester version of the oligonucleotide. When new targets within hTR were challenged with 2-5A-linked molecules, none showed any improvement over H1. We conclude that the H1 antisense molecule has the greatest specificity but that its efficacy is less if the backbone structure is changed either because of a reduced affinity for the target sequence or a reduced ability to activate RNaseL.			
14. SUBJECT TERMS Breast Cancer			15. NUMBER OF PAGES 5
			16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusions.....	5
References.....	N/A
Appendices.....	N/A

INTRODUCTION:

Telomerase is the riboprotein enzyme complex which prevents the ends of chromosomes from shortening below a critical length in cancer cells. This enzyme is normally not expressed in the majority of human cells after an early point in embryonic development but is reactivated in the vast majority (95%) of highly malignant cancer cells. It is thought to be an essential requirement for the maintenance of cell viability in cancer cells which express it. We have investigated whether inactivating telomerase in cancer cells using an antisense oligonucleotide approach targeting the RNA component of the enzyme will result in cell death. The oligonucleotides used carry a 2-5A moiety attached to the antisense molecule. 2-5A activates endogenous RNaseL which is normally found as an inactive monomer in the cytoplasm in most cells. In the presence of 2-5A the monomer dimerizes and becomes a potent RNase. Thus, the antisense molecule targets a specific RNA and the recruitment of RNaseL then selectively degrades the target. The overall aim of the project, therefore, is to determine whether inactivating telomerase can be developed as a viable form of anti cancer therapy for breast tumors. The initial series of experiments are designed to establish the conditions of treatment which will produce effective cell killing. In the last report we demonstrated that 2-5A-anti-hTR treatment of human breast cancer cell lines resulted in rapid cell death in vitro which was due to the induction of apoptosis. When tumors were induced in the flanks of nude mice and treated by direct injection of the antisense oligonucleotides, tumor development was retarded compared with control animals. We had thus demonstrated that growth of human breast cancer cell lines could be prevented by treating with an antisense oligonucleotide targeting the RNA component of telomerase.

BODY:

In October 2000 the P.I. accepted the position of Chair of Cancer Genetics at Roswell Park Cancer Institute and moved his laboratory there in November 2000. The USAMRMC was informed of this change of institution at that time and the paperwork to transfer the grant funding was submitted at the beginning of 2001. As of the 30th of September 2001 this transfer has still not taken place and no assurances that it will be completed could be obtained. As a consequence we have been unable to pursue any of the experimental objectives outlined in the statement of work for the final year of this grant. The outstanding tasks therefore remain as they did at the end of the second year.

- Task 1: Completed
- Task 2: Completed
- Task 3: In progress
- Task 4: Completed
- Task 5: Completed
- Task 6: Completed
- Task 7: Completed
- Task 8: Suspended due to the nuclease sensitivity of the oligos
- Task 9: Not initiated
- Task 10: Not initiated

We are in a position to undertake the analyses as described in the original application which is dependent on the transfer of the remaining funding for the project to hire personnel who will be dedicated to this task.

KEY RESEARCH ACCOMPLISHMENTS:

No progress due to the non-transfer of funding to the new Institution.

REPORTABLE OUTCOMES:

No progress due to the non-transfer of funding to the new Institution.

CONCLUSIONS:

No progress due to the non-transfer of funding to the new Institution.

REFERENCES:

None

APPENDICES

None